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(54) **Method of and apparatus for detecting the presence of live organisms in substances.**

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Description

This invention relates to a method of and apparatus for detecting the presence of live organisms in substances. A particular application of the invention is to the detection of the presence of bacteria in very small numbers in substances.

It is known that all or most live cells each contain a large number of ATP (andenosine-5'-triphosphate) molecules, many or all of which molecules are released if the cell is disrupted, that is to say, the outer cell membrane ruptured and the cell contents dispersed. (ATP molecules in dead cells are quickly broken down by autolysis). It is also known that when ATP molecules react with firefly essence (i.e. "Luciferin" reagent and "Luciferase" enzyme) photons of light are produced according to the following formula:

$$\text{Luciferin} + \text{ATP} + 0_2 \xrightarrow[\text{Magnesium ions}]{\text{Luciferase}} \text{light photons}$$

That is to say, the andenosine-5'-triphosphate ("ATP") molecules react with the reagent Luciferin in the presence of oxygen and magnesium ions and the Luciferase enzyme to produce light photons, one photon per ATP molecule. A living cell may contain approximately 100,000 ATP molecules. Reference may be made to an article entitled "The energy source for bioluminescence in an isolate system" by W. D. McElroy in Volume 33 (1974) page 342—345 of the Proceedings of the National Academy of Science and to another article entitled "luminometry—a sensitive technique in analytical chemistry and medical sciences" by S. Kolehmainen at pages 129—136 of the September/October 1979 issue of "International Laboratory".

GB—A—2 001 434 discloses a method of, and apparatus for, measuring somatic cells in a sample of milk by treating the cells to release ATP (molecules), mixing the sample with firefly luciferin-luciferase reagent and measuring the light intensity emitted by the sample. It suggests applications also to measurement of the biological activity of activated sludge in sewage treatment plants and of the biomass of aquatic organisms or soil microbes.

Even a single light photon may cause some types of photo-multiplier (PM) to produce a detectable electric pulse output. However, the PM has to be highly sensitive for this purpose, rendering the PM liable to produce electric pulse outputs in response to spurious disturbances as "noise". Furthermore, impurity ATP molecules react with the firefly essence to produce photons of light.

It is also known that a bacterium cell can be ruptured (or burst open) by violent agitation alone (e.g. ultrasonically) or with a cutting agent (if not ultrasonically) such as aluminium oxide, ballotini beads, sand r carborundum, in water.

The invention is based upon r stems from realisation or discovery of the fact that the act f disrupting a live cell (e.g. a bacterium) can be arranged t produce a "burst" f ph t ns (i. a substantial number of phot ns in a sh rt peri d of time) if the live cell is already in intimate contact with a substantial amount of firefly essence at the instant of distruption, so that all or substantially all of the ATP molecules which are released when the cell is disrupted react almost immediately with firefly essence to produce respective photons of light, and that this burst of photons can be distinguished from single stray photons and background noise. However, the inventive method and apparatus need not be limited to the reaction of ATP molecules with firefly essence.

According to a first aspect of the invention there is provided a method of detecting the presence of live organisms in a substance, comprising:— the step of acting upon the substance so as to cause disruption of the live organisms and hence release of a large number of molecules of a certain type from each disrupted organism; the step of mixing the substance with a reagent adapted to react with the released molecules in such a way as to cause emission of light photons; and the step of detecting the emission of said light photons; characterised in that the detected live organisms are a small number of cells; in that the step of mixing the substance with the reagent is carried out gently so as to avoid premature disruption of the live organisms; in that the step of acting upon the substance so as to cause the disruptions of said cells is carried out after the step of mixing the substance with the reagent, with the result that upon the release of the large number of said molecules from an individual cell, upon the disruption thereof, a burst of light photons is emitted due to substantially simultaneous reaction of the large number of the molecules with the reagent; and in that the step of detecting the emission of light photons is carried out in such a way that successive bursts of emitted light photons (indicative of distruptions of live organism cells) are distinguished from stray single emitted light photons (due to "noise" or impurity molecules of said certain type).

According to a second aspect of the invention there is provided apparatus for carrying out the method according to the first aspect of the invention, comprising:— means for acting upon the substance so as to cause disruption of the live organisms and hence release of a large number of molecules of a certain type as an individual organism is disrupted; a substantially transparent receptacle to contain the substance together with a reagent for reacting with the released molecules in such a way as to cause emission of light photons; a light-tight chamber for occupancy by the receptacle; means for mixing together the substance and the reagent; and means for detecting emission of light photons within the light-tight chamber fr m a mixture of the substance and the reagent in the substantially transparent receptacle; characterised in that:—

the mixing means is adapted to mix the substance and the reagent together gently in the receptacle, so as substantially t avoid premature disruption of the organisms; in that the means for acting upon the substances to cause the disruptions is adapted to act upon the mixture of the substance and the reagent in the receptacle, within the chamber, in such a way as to cause a disruption of a live organism cell relatively suddenly, so that said disruption results in the emission of a burst of light photons as the released molecules all react substantially simultaneously with the reagent; and in that the light photon emission detecting means is adapted for distinguishing successive bursts of emitted light photons (indicative of sudden distruptions of live organism cells) from stray single emitted light photons (due to "noise" or impurity molecules of said certain type).

The present invention is applicable particularly in situations where the substance contains very, very few live organisms, so few in fact that the stray single emitted light photons caused by impurity molecules of said certain type (for example, ATP molecules) might otherwise be mistakenly interrupted as evidence of live organisms.

The invention will be described by way of example with reference to the accompanying drawing, which illustrates an apparatus embodying the fourth aspect of the invention and adapted for use in a method in accordance with the first, second and third aspects of the invention.

Referring to the drawing, the illustrated apparatus 10 comprises a high speed vibratory tissue-disintegrator 11 which is a modification of one manufactured by Mickle Laboratory Engineering Co. Ltd. of Mill Works, Gomshall, Near Guildford, Surrey.

The tissue-disintegrator 11 comprises a single screw-capped metal container 12, for a transparent glass cuvette containing the substance being tested, mounted at an outer end of a vibratory reed 13. The container 12 has two window openings 14 facing along its axis 15 of vibration, that is to say, at right angles to the "plane" of the reed 13, for detection of photons of light inside the cuvette (which is faintly visible through one window opening 14) by two photomultipliers 16 which are mounted respectively opposite the two window openings 14. The other, inner, end of the reed 13 is clamped by a screw 17 to a firmly mounted base 18. Upon loosening the screw 17, the effective length of the reed 13 can be altered by pushing it into or pulling it out of the base 18. The container 12 and reed 13 are counterbalanced relative to the base 18 when vibrating by a second reed 19 carrying counterweights 20 and clamped to the base 18 by a second screw 21, upon loosening which the effective length of reed 19 can be varied, analogously to the reed 13. Two electromagnets 22 and 23 are fixedly mounted (by means not shown) adjacent intermediate portions of reeds 13 and 19 respectively and are energisable by alternating r pulsating electric current to vibrate the reeds 13 and 19 respectively. Finally, the tissue-distintegrator 11 comprises a light-tight box 24, indicated by broken lines, containing items 12 to 23, so that the only light to reach photomultipliers 16 is that from inside the cuvette in operation as described hereinafter.

The apparatus 10 also comprises electronic circuitry 25 for processing the outputs from the photomultipliers 16 (and for controlling the vibration of reeds 13 and 19 and hence of container 12).

The electronic circuitry 25 comprises the following components:— a high voltage source 26 connected to the two photomultipliers 16 as shown to supply them with high voltage; two amplifiers 27 respectively connected to the outputs of the two photomultipliers 16 as shown to amplify their respective outputs; two adjustable signal attenuators 28 connected to the outputs of the two amplifiers 27 to adjustably attenuate their outputs for, inter alia, the purpose of distinguishing between genuine signals and noise; (these may be replaced by threshold devices such as Zener diodes, not shown); and a two-gang, two-pole changeover switch 29 connected to the outputs of attenuators 28.

For a preferred mode of operation, the electronic circuitry 25 also comprises: a fast counter 30 for cumulatively counting output pulses from the two attenuators 28 (or the alternative threshold devices mentioned above); a fast timer 31 for timing a selected short interval, of the order of, for example, one millisecond, from the arrival of a pulse at counter 30 when counter 30 is at zero, and for resetting counter 30 to zero at the end of said short interval; an interval selector 32 for selecting the short interval to be timed by timer 31; a count selector 33 for selecting a minimum count of counter 30 (in the short interval timed by timer 31) to produce an "event" pulse to pass via selector 33 from counter 30 to an event counter 34; a "slow" timer 35 for timing a interval for the event counter 34, for determining the number of "events" in this interval of time (and then optionally resetting counter 34 to zero); and a display unit 36 for displaying the number of events counted by counter 34. A "chemiluminescence delay ratemeter" 37 controls via outputs indicated schematically at 37a, the energisation of electromagnets 22 and 23 and hence the vibration of container 12, to produce selectively nil agitation, gentle agitation and violent agitation of the cuvette. The chemiluminescence delay ratemeter 37 (referred to as the "ratemeter 37" for brevity) receives an input from counter 30, whereby abnormally high counts during gentle agitation, or even no agitation, can be detected. At this point it is apposite to explain that the gentle agitation is intended to produce good mixing of the substance and the reagent but without disruption of any live cells, so that abnormally high counts would normally be due t impurities in the reagent or even in the substance.

Accordingly the ratemeter 37 is responsiv to abnormally high counts during n agitation or gentle agitati n respectively to delay commencement of gentle agitation or violent agitation until the counts have subsided to a normal level. Ratemeter 37 is connected to a recorder 38 for recording results of tests.

For an alternative mode of operation, the electronic circuitry 25 also comprises: a coincidence detector 39 for detecting simultaneous or substantially simultaneous output pulses from the two attenuators 28 (or the alternative threshold devices mentioned above); a summation amplifier 40 for summing the pulses detected as coincident by detector 39; a counter display 41 for displaying the result; and a timer 42 whose function is analogous to that of timer 35.

In use, in for example a test for the presence of any living cells in a substance, a sample of the substance is put into a transparent cuvette of known type. Also put into the cuvette is firefly essence (i.e. Luciferin and Luciferase) in substantial excess, an aqueous buffer solution (for the oxygen and magnesium ions) and a cutting agent such as aluminium oxide. The cuvette is then placed in the container 12. The lengths of the arms 13 and 19 will already have been adjusted for resonant vibration at the frequency of the supply voltage. Then, with switch 29 set to connect attenuators 28 (or the alternative threshold devices mentioned above) to counter 30, with interval selector 32 set at about one millisecond, and count selector 33 set at five (that is, a count of five pulses in counter 30 in the one millisecond interval set by selector 32 to constitute an "event") the apparatus is switched on. Initially there is no vibration of container 12. If it should happen that stray ATP molecules and other "noise" factors cumulatively are producing an abnormally high pulse rate from counter 30 (that is, substantially higher than the normal rate of, say, one per millisecond on average, the ratemeter 37 indicates this and postpones the onset of agitation of the container 12. When the pulse rate from counter 30 has fallen to a normal level, which happens when the stray ATP molecules have nearly all reacted with the firefly essence, the ratemeter 37 switches on a low voltage supply to electromagnets 22 and 23 for gentle agitation of the cuvette in container 12, to achieve thorough pre-mixing without (substantial) disruption of any live cells therein. This gentle agitation is carried out until stray ATP molecules and other noise factors, introduced as a consequence of gentle agitation, cumulatively are producing a normal pulse rate from counter 30 as determined by the ratemeter 37, after which the ratemeter 37 switches the energisation of electromagnets 22 and 23 from low to high voltage and starts timer 36. The high voltage causes violent agitation of the cuvette so that the cutting agent disrupts or burst open live cells in the mixture, releasing ATP molecules which quickly encounter and react with the firefly essence (which is there in excess) to produce a burst or bursts of light photons which are detected by the photomultipliers 16. It may be assumed that, given the fact that appr ximately 100,000 ATP molecules are released when a live cell is disrupted, there will be a certain time spread in the production of the photons, instead of all the photons being produced simultaneously, so that at least, say, five distinct pulses will be produced by counter 30 in response to at least five distinct photons or groups of photons, for "event counter" 34 to count an "event", namely, the disruption (and thus the presence) of a live cell, (live, that is, until killed by the violent agitation in the conditions set out above). The number of "events" indicated on display unit 36 is equal to, or at least indicative of, the number of live cells present in the sample in the cuvette (not shown) in container 12 before the apparatus was switched on.

The following comments may be made about the above-described apparatus and method:—

i) the ability of ratemeter 37 to delay the onset of violent agitation (and even the onset of gentle agitation) if the pulse rate is abnormally high during gentle agitation (or during no agitation respectively) allows impurity ATP molecules to react with the Firefly essence before measurements start, so as not to affect the measurements themselves. Hence it is possible to use poor quality reagents containing impurity ATP or possible to test samples containing impurity ATP molecules;

ii) because the cells are already intimately mixed with the firefly essence when they become disrupted, the ATP molecules and the firefly essence react together almost immediately;

iii) because violent agitation is taking place during cell disruption, the ATP molecules can come into contact with fresh firefly essence very quickly, even if the firefly essence in the immediate vicinity of the cell at the time of disruption is exhausted by reaction with other ATP molecules;

iv) the arrangement is designed to give the maximum light output in the shortest possible time for each disruption of a living organism cell;

v) the essence of the method is that by keeping the living organism cells intact in the cuvette until after they have been mixed with an exces of firefly essence, and then rupturing the cells in the presence of the excessive firefly essence the light produced by each cloud of ATP molecules from individual cells reacting with the firefly essence appears as a burst of light, related to the time of rupturing, these bursts of light being detected electronically as "events";

vi) this method permits the detection of a very small number of living organism cells, namely, approximately ten per millilitre, in a very short time, in fact, less than ten seconds;

vii) very little preparation of each sample is required, so that there is the possibility f further development of the invention to incorporate an automatic sample charger for the purpose of

sequential automatic testing of a number of different samples;

viii) the choice f one millisecond for the fast timer 31 and a counter of five for the count selector 33 is based on estimation of the probability that a count of five photon-produced pulses in one millisecond is unlikely except in the event of rupture of a living organism cell. It is possible that certain substances and/or certain qualities of firefly essence will require modification of these settings.

For the alternative mode of operation mentioned above, using items 39 to 42 of the electronic circuitry 25, with switch 29 switched over to connect the attenuators 28 (or the alternative threshold devices mentioned above) to coincidence detector 39 and summation amplifier 40, the method relies upon the probability that only rupture of a living organism cell will produce sufficient light to cause production substantially simultaneously of output pulses from both photomultipliers 16.

It is conceivable that some other method, such as ultrasonic agitation, might be used for rupturing the cells, instead of the vibratory tissue disintegrator 11.

Furthermore, it may be deemed desirable to cool the cuvette or to maintain a constant temperature of the cuvette during measurement, and/or to do likewise with the photomultipliers 16.

It may be desirable to repeat the measureemnt after a period of incubation to allow cell multiplication in the cuvette.

It may be desirable to add nutrient media, and/or other reagents and chemicals to the mixture in the cuvette, prior to measurement.

Other light detecting devices besides photomultipliers, for example, silicone photodiodes, may be used.

Furthermore, in the preferred mode of operation, it is possible that a single light detector (whether photomultiplier or other) may be sufficient, or that more than two light detectors may be desirable.

It is possible that light losses due to absorption, low photon detection efficiency of the ligth detectors, excessive photon production by impurity ATP molecules, and electronic noise during the actual cell measurement, may require a much higher setting of the count selector 33, such that substantially simultaneous distruption of two, three or even more living organism cells is necessary for an "event" to be detected.

Claims

1. A method of detecting the presence of live organisms in a substance, comprising:— the step of acting upon the substance so as to cause disruption of the live organisms and hence release of a large number of molecules of a certain type from each disrupted organism; the step of mixing the substance with a reagent adapted to react with the released molecules in such a way as to cause emission of light photons; and the step of detecting the emission of said light photons; characterised in that the detected live organisms are a small number of cells; in that the step of mixing the substance with the reag nt is carried out gently so as to avoid premature disruption of the live organisms; in that the step of acting upon the substance so as to cause the disruptions of said cells is carried out after the step of mixing the substance with the reagent, with the result that upon the release of the large number of said molecules from an individual cell, upon the disruption thereof, a burst of light photons is emitted due to substantially simultaneous reaction of the large number of the molecules with the reagent; and in that the step of detecting the emission of light photons is carried out in such a way that successive bursts of emitted light photons (indicative of disruptions of live organism cells) are distinguished from stray single emitted light photons (due to "noise" or impurity molecules of said certain type).

2. A method as claimed in Claim 1 wherein the molecules are ATP molecules and the reagent is firefly essence.

3. A method as claimed in claim 1 or 2 wherein the disruption is caused by violent agitation.

4. A method as claimed in claim 3 wherein the disruption is caused by violent agitation with a disrupting agent.

5. A method as claimed in claim 4 wherein the disrupting agent comprises aluminium oxide, ballotini beads, sand or carborundum.

6. A method as claimed in any preceding claim wherein each detected photon produces an electrical pulse output from a photon detector.

7. A method as claimed in claim 6 wherein a timed period is commenced in response to an electrical pulse from the photon detector and the number of electrical output pulses from the detector in the timed period is counted by a fast counter, to be recorded as a single event in a slow counter if said number exceeds a threshold value.

8. A method as claimed in any preceding claim wherein spurious signal outputs due to "noise" or impurity molecules of said certain type are distinguished from a burst of photons produced upon disruption of a live organism or a number of organisms by counting the photons detected in a short time period commencing with an initial detected photon, and recording the count as (at least) one disrupted live organism if the count is above a predetermined minimum.

9. A method as claimed in any one of claims 1 to 7 wherein a plurality of spatially distributed detectors are used to detect photons and wherein only substantially simultaneous outputs from all or at least some of said detectors are counted as a disrupted live organism.

10. Apparatus for carrying out the method of Claim 1, comprising:— means (13, 22) for acting upon the substance so as to cause disruption of the live organisms and hence release of a large number of molecules of a certain type as an individual organism is disrupted; a substantially transparent receptacle to contain the substanc s

together with a reagent for reacting with the released molecules in such a way as to cause emission f light photons; a light-tight chamber for occupancy by the receptacle; means (13, 22) for mixing together the substances and the reagent; and means (16, 25) for detecting emission of light photons within the light-tight chamber from a mixture of the substance and the reagent in the substantially transparent receptacle; characterised in that:— the mixing means (13, 22) is adapted to mix the substance and the reagent together gently in the receptacle, so as substantially to avoid premature disruption of the organisms; in that the means (13, 22) for acting upon the substance to cause the disruptions is adapted to act upon the mixture of the substance and the reagent in the receptacle, within the chamber, in such a way as to cause a disruption of a live organism cell relatively suddenly, so that said disruption results in the emission of a burst of light photons as the released molecules all react substantially simultaneously with the reagent; and in that the light photon emission detecting means (16, 25) is adapted for distinguishing successive bursts of emitted light photons (indicative of sudden disruptions of live organism cells) from stray single emitted light photons (due to "noise" or impurity molecules of said certain type).

11. Apparatus as claimed in Claim 10 wherein the mixing means and the means for acting upon the substance comprise means (13, 22) for vibrating the receptacle selectively either gently (for mixing) or violently (for cell-disruption), within the chamber.

12. Apparatus as claimed in Claim 10 or 11 wherein the mixing means and/or the means for acting upon the substance comprises a vibratory receptacle-holding member (12, 13) and an electromagnet (22) to produce the vibration.

13. Apparatus as claimed in Claim 10, 11 or 12 wherein the photon-detecting means comprises means (37) for detecting excessive spurious photons and for delaying the organism-disrupting action until after the intensity of the spurious photons has fallen to a predetermined acceptable level.

14. Apparatus as claimed in any one of claims 10 to 13 wherein a timer (31) is responsive to an electrical output pulse from the photon-detecting means to activate a fast counter (30) to count the number of output pulses from the photon-detecting means (16) in a short time period and thereafter to reset the fast counter (30) to zero, and a slow counter (34) is responsive to the count in the fast counter (30) exceeding a threshold value in any such time period to count a live organism.

Revendications

1. Procédé pour détecter la présence d'organismes vivants dans une substance, incluant: la phase opératoire consistant à agir sur la substance de manière à provoquer la destruction des organismes vivants, et par c nséquent la libération d'un grand nombre de molécules d'un certain type à partir de chaque rganisme détruit; la phase opératoire de mélange de la substance avec un réactif apte à réagir avec les molécules libérées de manière à provoquer l'émission de photons; et la phase opératoire consistant à détecter l'émission desdits photons; caractérisé en ce que les organismes vivants détectés sont un petit nombre de cellules; que la phase opératoire de mélange de la substance avec le réactif est mise en oeuvre en douceur de manière à éviter une destruction prématurée des organismes vivants; que la phase opératoire consistant à agir sur la substance de manière à provoquer les destructions desdites cellules est mise en oeuvre après la phase opératoire de mélange de la substance avec le réactif, avec pour résultat que lors de la libération du nombre important desdites molécules par une cellule individuelle, lors de sa destruction, une rafale de photons est émise par suite d'une réaction sensiblement simultanée du nombre élevé des molécules avec le réactif; et que la phase opératoire de détection de l'émission des photons est mise en oeuvre de telle manière que les rafales successives de photons émis (ce qui est l'indication de destructions de cellules d'organismes vivants) sont différenciées des photons parasites émis isolément (dûs à un "bruit" ou à des molécules d'impuretés d'un certain type).

2. Procédé selon la revendication 1, selon lequel les molécules sont des molécules ATP et le réactif est à base d'extraits de luciole.

3. Procédé selon la revendication 1 ou 2, selon lequel la destruction est provoquée par une agitation violente.

4. Procédé selon la revendication 3, selon lequel la destruction est provoquée par une agitation violente à l'aide d'un agent de destruction.

5. Procédé selon la revendication 4, selon lequel l'agent de destruction comprend de l'oxyde d'aluminium, des couches de ballotines, du sable ou carborundum.

6. Procédé selon l'une quelconque des revendications précédentes, selon lequel chaque photon détecté produit une impulsion électrique délivrée par un détecteur de photons.

7. Procédé selon la revendication 6, selon lequel une période chronométrée est déclenchée en réponse à une impulsion électrique délivrée par le détecteur de photons, et le nombre des impulsions électriques de sortie du détecteur pendant la période chronométrée est compté par un compteur rapide afin d'être enregistré sous la forme d'un évènement unique dans un compteur lent, si ledit nombre dépasse une valeur de seuil.

8. Procédé selon l'une quelconque des revendications précédentes, selon lequel des sorties de signaux parasites, dûs à un "bruit" ou à des molécules d'impuretés d'un certain type, sont différenciées d'une rafale de photons produite lors de la destruction d'un organisme vivant ou d'un certain nombre d'organismes, au moyen du comptage des photons détectés pendant un inter-

valle de temps de brève durée commençant lors de la première détection d'un photon, et grâce à l'enregistrement du nombre compté commen représentant (au moins) un organisme vivant détruit, si le nombre compté dépasse un minimum prédéterminé.

9. Procédé selon l'une quelconque des revendications 1 à 7, selon lequel une pluralité de détecteurs répartis dans l'espace sont utilisés pour détecter les photons, et selon lequel uniquement des signaux de sortie sensiblement simultanés provenant de l'ensemble ou d'au moins une partie desdits détecteurs sont comptés comme représentant un organisme vivant détruit.

10. Appareil pour la mise en oeuvre du procédé selon la revendication 1, comprenant: des moyens (13, 22) servant à agir sur la substance de manière à provoquer la destruction des organismes vivants et par conséquent la libération d'un nombre important de molécules d'un certain type lorsqu'un organisme individuel est détruit; un réceptacle essentiellement transparent servant à contenir les substances ainsi qu'un réactif servant à réagir avec les molécules libérées de manière à provoquer l'émission de photons; une chambre étanche à la lumière prévue pour recevoir le réceptacle; des moyens (13, 22) pour mélanger ensemble les substances et le réactif; et des moyens (16, 25) pour détecter l'émission de photons à l'intérieur de la chambre étanche à la lumière, à partir d'un mélange de la substance et du réactif dans le réceptacle essentiellement transparent; caractérisé en ce que: les moyens de mélange (13, 22) sont aptes à mélanger la substance et le réactif de façon douce à l'intérieur du réceptacle, de manière à éviter pour l'essentiel une destruction prématurée des organismes; que les moyens (13, 22) servant à agir sur la substance en vue de provoquer les destructions sont adaptés pour réagir sur le mélange de la substance et du réactif situés dans le réceptacle, à l'intérieur de la chambre, de manière à provoquer d'une manière relativement subite la destruction d'une cellule d'un organisme vivant, de telle sorte que ladite destruction provoque l'émission d'une rafale de photons lorsque les molécules libérées réagissent toutes essentiellement simultanément avec le réactif; et que les moyens (16, 25) de détection de l'émission des photons sont adaptés pour différencier des rafales successives de photons émis (indiquant des destructions subites des cellules de l'organisme vivant) par rapport à des photons parasites émis individuellement (dûs à un "bruit" ou à des molécules d'impuretés d'un certain type).

11. Appareil selon la revendication 10, dans lequel les moyens de mélange et les moyens permettant d'agir sur la substance comprennent des moyens (13, 22) pour mettre en vibrations le réceptacle au choix soit selon un mode doux (pour le mélange), soit selon un mode violent (pour la destruction des cellules) à l'intérieur de la chambre.

12. Appareil selon la revendication 10 ou 11, dans lequel les moyens de mélange et/ou les moyens servant à agir sur la substance comprennent un organe vibrant (12, 13) maint nant le réceptacle, et un électroaimant (22) servant à produire les vibrations.

13. Appareil selon la revendication 10, 11 ou 12, dans lequel les moyens de détection des photons comprennent les moyens (37) servant à détecter des photons parasites en excès, et servant à retarder l'action de destruction des organismes jusqu'à ce que l'intensité des photons parasites soit tombée à un niveau acceptable prédéterminé.

14. Appareil selon l'une quelconque des revendications 10 à 13, dans lequel une minuterie (31) répond à une impulsion électrique de sortie délivrée par les moyens de détection des photons de manière à activer un compteur rapide (30) afin de compter le nombre des impulsions de sortie provenant des moyens (16) de détection des photons, pendant un bref intervalle de temps, et ensuite pour ramener à zéro le compteur rapide (30), et un compteur lent (34) répond au fait que le nombre compté dans le compteur rapide (30) dépasse une valeur de seuil, pendant une quelconque période chronométrée pour compter un organisme vivant.

Patentansprüche

1. Verfahren zum Feststellen der Anwesenheit von lebenden Organismen in einer Substanz mit den Schritten: Einwirken auf die Substanz zum Zwecke des Zerbrechens der lebenden Organismen und folglich Freilassens einer großen Zahl von Molekülen eines bestimmten Typs aus jedem zerbrochenen Organismus, Mischun der Substanz mit einem Reagenz, das auf eine Reaktion mit den freigelassenen Molekülen derart ausgerichtet ist, daß es die Emission von Lichtquanten verursacht, und Feststellen der Emission der genannten Lichtquanten, dadurch gekennzeichnet, daß die festgestellten lebenden Organismen eine kleine Zahl von Zellen sind, daß das Mischen der Substanz mit dem Reagenz zur Vermeidung eines vorzeitigen Zerbrechens der lebenden Organismen sanft ausgeführt wird, daß die Einwirkung auf die Substanz zum Bewirken des Zerbrechens der genannten Zellen nach dem Mischen der Substanz mit dem Reagenz stattfindet mit dem Ergebnis, daß infolge Freilassung der großen Zahl der genannten Moleküle aus einer einzelnen Zelle durch deren Zerbrechen ein Schauer von Lichtquanten emitiert wird wegen der im wesentlichen gleichzeitigen Reaktion der großen Zahl der Moleküle mit dem Reagenz, und daß die Feststellung der Emission der Lichtquanten auf solche Weise durchgeführt wird, daß aufeinanderfolgende Schauer emitierter Lichtquanten (Indikatoren für Zerstörungen von Zellen lebender Organismen) unterschieden sind von einzeln vorstreut emitierten Lichtquanten (verursacht durch "Rausch-" oder Verunreinigungsmoleküle des genannten bestimmten Typs).

2. Verfahren nach Anspruch 1, dadurch gekenn-

zeichnet, daß die Moleküle ATP-Moleküle sind und daß das Reagenz Leuchtkäf-eressenz ist.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß das Zerbrechen durch heftiges Schütteln bewirkt wird.

4. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß das Zerbrechen durch heftiges Schütteln mit einem Berstmittel bewirkt wird.

5. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß das Berstmittel Aluminiumoxid, Glaskügelchen, Sand oder Kar/borund enthält.

6. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß jedes festgestellte Lichtquant die Ausgabe eines elektrischen Impulses durch einen Photonendetektor bewirkt.

7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß als Reaktion auf einen elektrischen Impuls des Photonendetektors eine zeitlich festgelegt Periode begonnen wird und daß die Zahl der elektrishcen Ausgangsimpulse des Detektors innerhalb der zeitlichen festgelegten Impulse durch einen schnellen Zähler gezählt wird, um als ein einzelnes Ereignis in einem langsamen Zähler festgehalten zu werden, wenn die genannte Zahl einen Schwellenwert überschreitet.

8. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die infolge "Rausch-" oder Verunreinigungsmolekülen des genannten bestimmten Typs ausgegebenen Störsignale unterschieden werden von einem Schauer von durch Zerbrechen eines lebenden Organismus oder einer Zahl von Organismen erzeugten Lichtquanten mittels Zählens der festgestellten Lichtquanten innerhalb einer kurzen, mit einem anfänglichen festgestellten Lichtquant beginnenden Zeitperiode und Registrierens der Zahl als (wenigstens) eine zerbrochenen lebenden Organismus, wenn die Zahl oberhalb eines vorherbestimmten Minimums liegt.

9. Verfharen nach einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, daß eine Mehrzahl räumlich verteilter Detektoren zur Feststellung von Lichtquanten verwendet wird und daß nur im wesentlichen gleichzeitige Ausgaben aller oder wenigstens einiger der genannten Detektoren als ein zerbrochener lebender Organismus gezählt werden.

10. Vorrichtung zur Durchführung des Verfahrens nach Anspruch 1, enthaltend Mittel (13, 22) zur Einwirkung auf die Substanz mit dem Ziel eines Zerbrechens der lebenden Organismen und infolgedessen Freilassens einer großen Zahl von Molekülen eines bestimmten Typs, wenn ein einzelner Organismus zerbrochen ist, ein im wesentlichen durchsichtiges Behältnis zur Aufnahme der Substanzen zusammen mit einem Reagenz für die Reaktion mit den freigelassenen Molekülen derart, daß die Emission von Lichtquanten verursacht wird, eine lichtdichte Kammer für die Aufnahme des Behältnisses, Mittel (13, 22) zum Zusammenmischen der Substanzen und des Reagenz und Mittel (16, 25) für die Feststellung der Emission von Lichtquanten innerhalb der lichtdichten Kammer aus einer Mischen der Substanz und des Reagenz innerhalb des im wesentlichen durchsichtigen Behältnisses, dadurch gekennzeichnet, daß die Mittel (13, 22) zum Mischen ausgerichtet sind auf ein sanftes Zusammenmischen der Substanz und des Reagenz in dem Behältnis im wesentlichen zur Vermeidung eines vorzeitigen Zerbrechens der Organismen, daß die Mittel (13, 22) zur Einwirkung auf die Substanz zur Herbeiführung der Zerbrechungen ausgerichtet sind auf eine Einwirkung auf die Mischung der Substanz und des Reagenz in dem Behältnis, das sich in der Kammer befindet, auf solche Weise, daß ein verhältnismäßig plötzliches Zerbrechen einer Zelle eines lebenden Organismus bewirkt wird, so daß das genannte Zerbrechen zur Emission einer Schauer von Lichtquanten führt, wenn die freigelassenen Moleküle alle im wesentlichen gleichzeitig mit dem Reagens reagieren, und daß die Mittel (16, 25) zur Feststellung der Lichtquantenemission eingerichtet sind auf die Unterscheidung aufeinanderfolgender Schauern emitierter Lichtquanten (Indikatoren plötzlichen Zerbrechens der Zellen lebender Organismen) von einzeln verstreut emitierten Lichtquanten (verursacht durch "Rausch-" oder Verunreinigungsmoleküle des genannten bestimmten Typs).

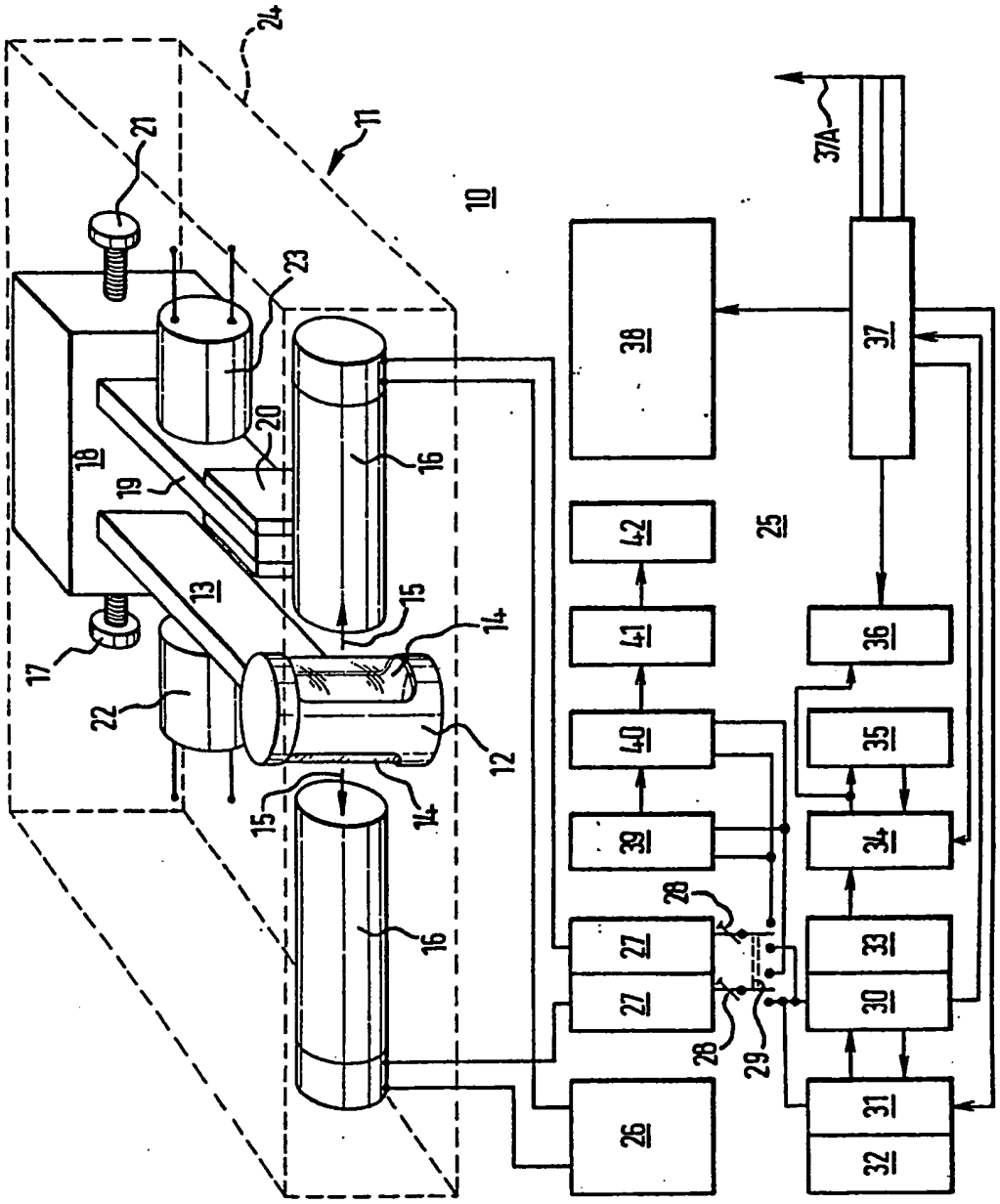
11. Vorrichtung nach Anspruch 10, dadurch gekennzeichnet, daß die Mittel zum Mischen und die Mittel zur Einwirkung auf die Substanz Mittel (13, 22) für die Vibration des in der Kammer enthaltenen Behältnisses entweder in sanfter Weise (zum Mischen) oder in heftiger Weise (zum Zerbrechen der Zellen) enthalten.

12. Vorrichtung nach Anspruch 10 oder 11, dadurch gekenzeichnet, daß die Mittel zum Mischen und/oder die Mittel zur Einwirkung auf die Substanz ein vibrierendes Teil (12, 13) für die Halterung des Behältnisses und einen Elektromagneten (22) zur Erzeugung der Vibration enthalten.

13. Vorrichtung nach Anspruch 10, 11 oder 12, dadurch gekennzeichnet, daß die Mittel zur Feststellung der Lichtquanten Mittel (37) für die Feststellung übermäßiger Störphotonen und für die Verschiebung der Einwirkung zum Zerbrechen der Organismen bis zu einem Zeitpunkt enthalten, nach dem die Intensität der Störphotonen auf einen vorherbestimmten, annehmbaren Wert gefallen ist.

14. Vorrichtung nach einem der Ansprüche 10 bis 13, dadurch gekennzeichnet, daß ein Zeitgeber (31) auf einen elektrischen Ausgangsimpuls der Mittel zum Feststellen der Lichtquanten reagiert, um einem schnellen Zähler (30) zur Zählung der Zahl der Ausgangsimpulse der Mittel (16) zur Feststellung der Lichtquanten innerhalb eines kurzen Zeitintervalls zu aktivieren und

danach den schnellen Zähler (30) auf null zurückzustellen, und daß ein langsamer Zähler (34) auf die Zahl des schnellen Zählers (30) reagiert, die einen Schwellenwert in einem solchen Zeitintervall übersteigt, um einen lebenden Organismus zu zählen.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